US007964078B2

(12) **United States Patent**
Lee et al.

(10) **Patent No.: US 7,964,078 B2**
(45) **Date of Patent: Jun. 21, 2011**

(54) **MICROFLUIDIC DEVICE FOR CELL AND PARTICLE SEPARATION**

(75) Inventors: **Abraham P. Lee**, Irvine, CA (US); **Lisen Wang**, Irvine, CA (US); **Lisa Flanagan Monuki**, Irvine, CA (US); **Edwin S. Monuki**, Irvine, CA (US); **Svyatoslav A. Marchenko**, North Hollywood, CA (US)

(73) Assignee: **The Regents of the University of California**, Oakland, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1175 days.

(21) Appl. No.: **11/670,945**

(22) Filed: **Feb. 2, 2007**

(65) **Prior Publication Data**

US 2007/0125941 A1 Jun. 7, 2007

Related U.S. Application Data

(63) Continuation-in-part of application No. 11/557,060, filed on Nov. 6, 2006.

(60) Provisional application No. 60/743,220, filed on Feb. 2, 2006.

(51) **Int. Cl.**
B03C 5/02 (2006.01)

(52) **U.S. Cl.** **204/643**; 204/547

(58) **Field of Classification Search** 204/547, 204/643

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,146,103 A * 11/2000 Lee et al. 417/50
6,936,811 B2 8/2005 Kibar
2002/0088712 A1 * 7/2002 Miles 204/547
2004/0091392 A1 * 5/2004 McBride et al. 422/57
2005/0072677 A1 * 4/2005 Gascoyne et al. 204/547

OTHER PUBLICATIONS

Yang, Jun, et al., "Cell Separation on Microfabricated Electrodes Using Dielectrophoretic/Gravitational Field-Flow Fractionation", Anal. Chem. vol. 71, No. 5, Mar. 1, 1999, pp. 911-918.
Fu, Anne Y., et al., "A Microfabricated Fluorescence-Activated Cell Sorter", Nature Biotechnology, vol. 17, Nov. 1999, pp. 1109-1111.
Heida, Tjitske, et al., "Dielectrophoretic Trapping of Dissociated Fetal Cortical Rat Neurons", IEEE Transactions on Biomedical Engineering, vol. 48, No. 8, Aug. 2001, pp. 921-930.

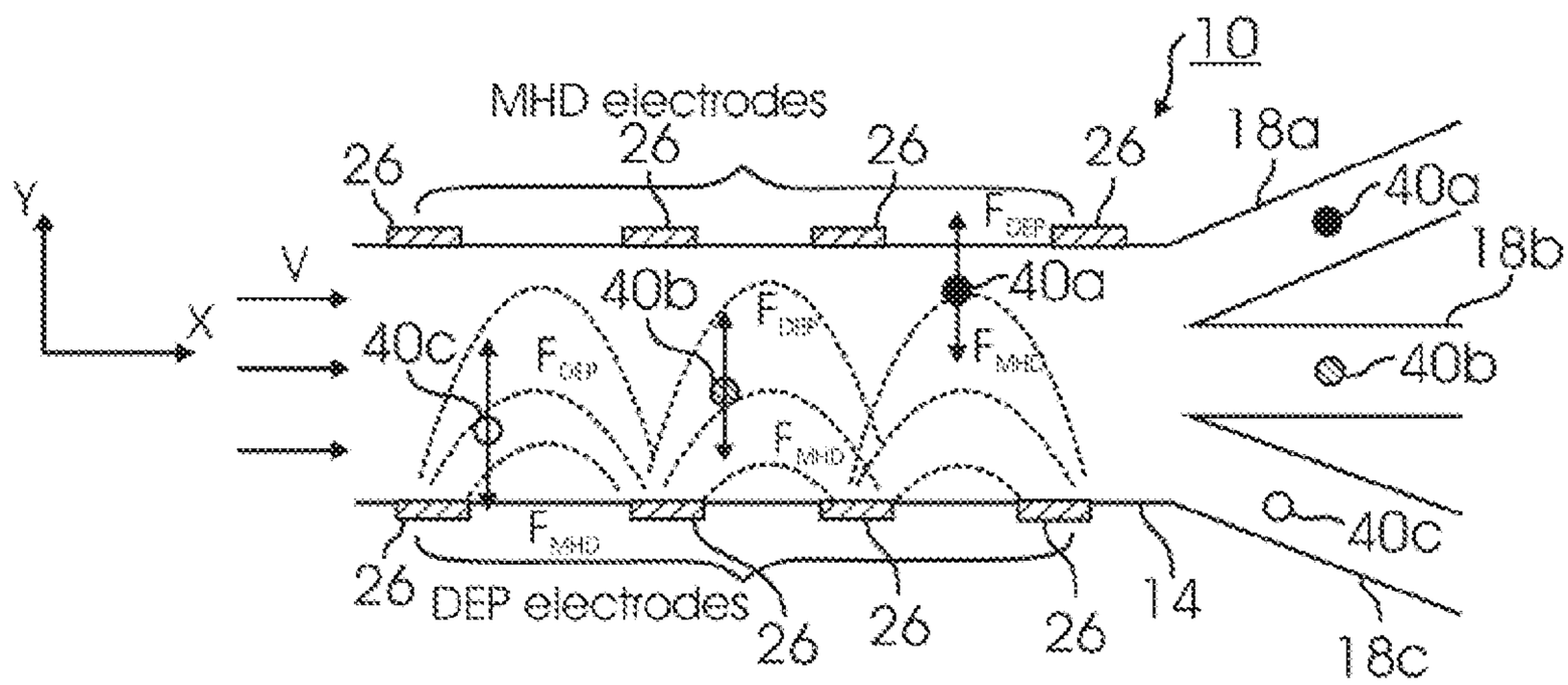
(Continued)

Primary Examiner — Ula C Ruddock
Assistant Examiner — J. Christopher Ball
(74) *Attorney, Agent, or Firm* — Vista IP Law Group LLP

(57) **ABSTRACT**

A microfluidic separation device includes a microchannel formed in a substrate and being defined at least by a bottom surface, a first side wall, and second side wall. Fluid containing particles or cells is flowed through the microchannel from an upstream end to a downstream end. The downstream end terminates in a plurality of branch channels. A plurality of vertically-oriented electrodes are disposed on the first wall and on the second wall opposite to the first wall. A voltage source is connected to the plurality of opposing electrodes to drive the electrodes. The opposing, vertically-oriented electrodes may be used to focus a heterogeneous population of particles or cells for subsequent downstream separation via additional electrodes placed on one of the side walls. Alternatively, the opposing, vertically-oriented electrodes may be used to spatially separate a heterogeneous population of particles or cells for later collection in one or more of the branch channels.

15 Claims, 7 Drawing Sheets



OTHER PUBLICATIONS

Lemoff, A.V., et al., "An AC Magnetohydrodynamic Microfluidic Switch", U.S. Department of Energy, Lawrence Livermore National Laboratory, UCRL-JC-137192, MicroTAS 2000, The Netherlands, May 14-18, 2000 [6pp].

Taff, B.M., et al., "A Scalable Row/Column-Addressable Dielectrophoretic Cell-Trapping Array", Massachusetts Institute of Technology, USA, 9th International Conference on Miniaturized Systems for Chemistry and Life Sciences, Oct. 9-13, 2005, Boston, Massachusetts, USA, pp. 865-867.

* cited by examiner

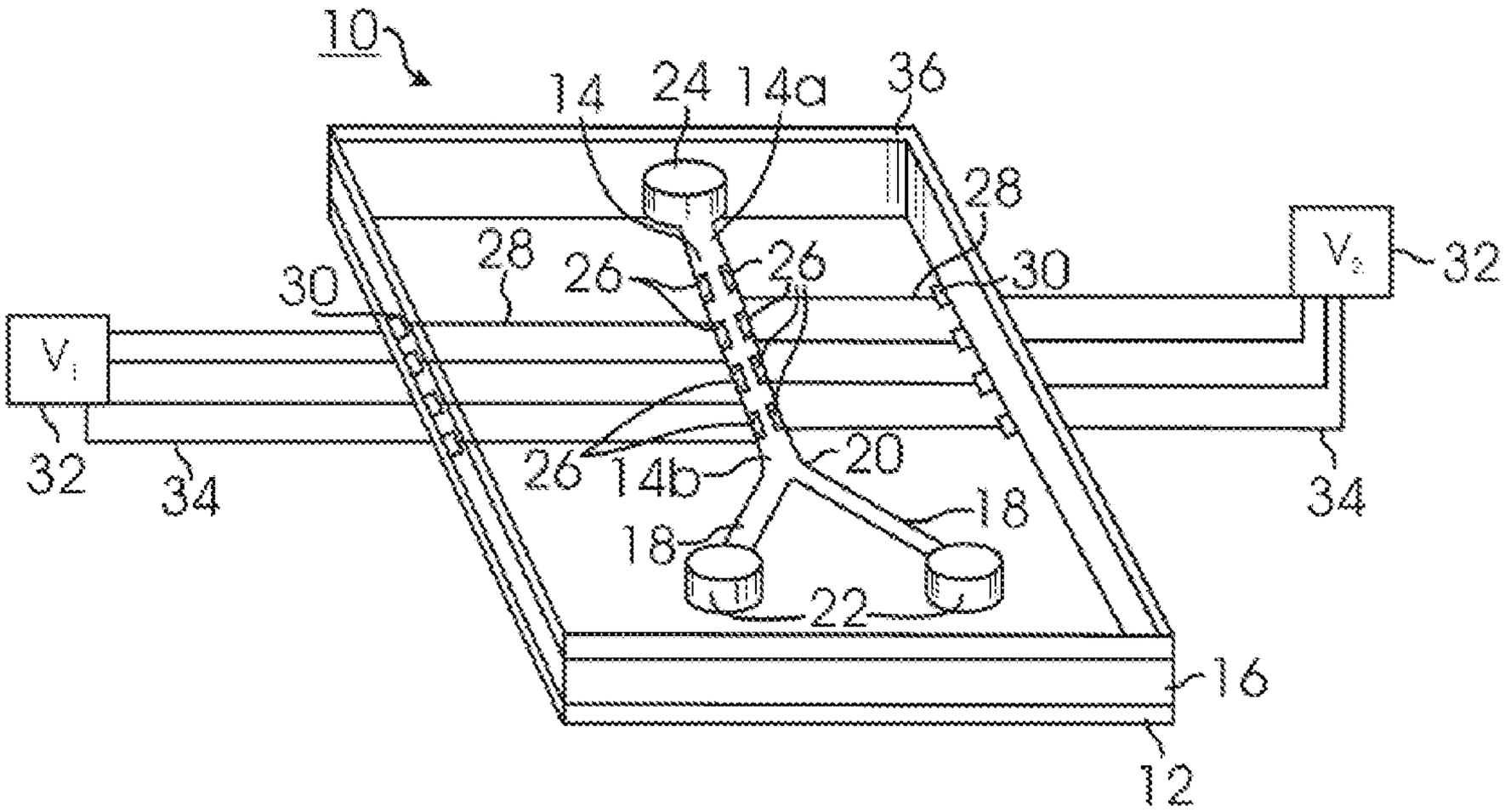

10
14
24
14a
36
28
28
26
26
30
30
V_s
32
V_i
32
34
26
14b
20
18
18
34
22
16
12

FIG. 1

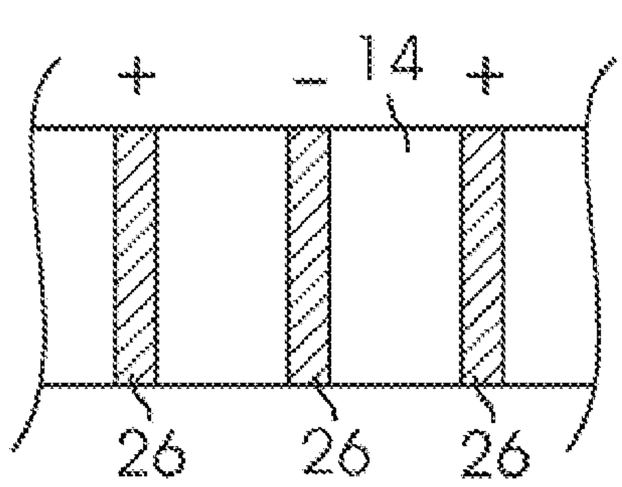

+
−
14
+
26
26
26

FIG. 2A

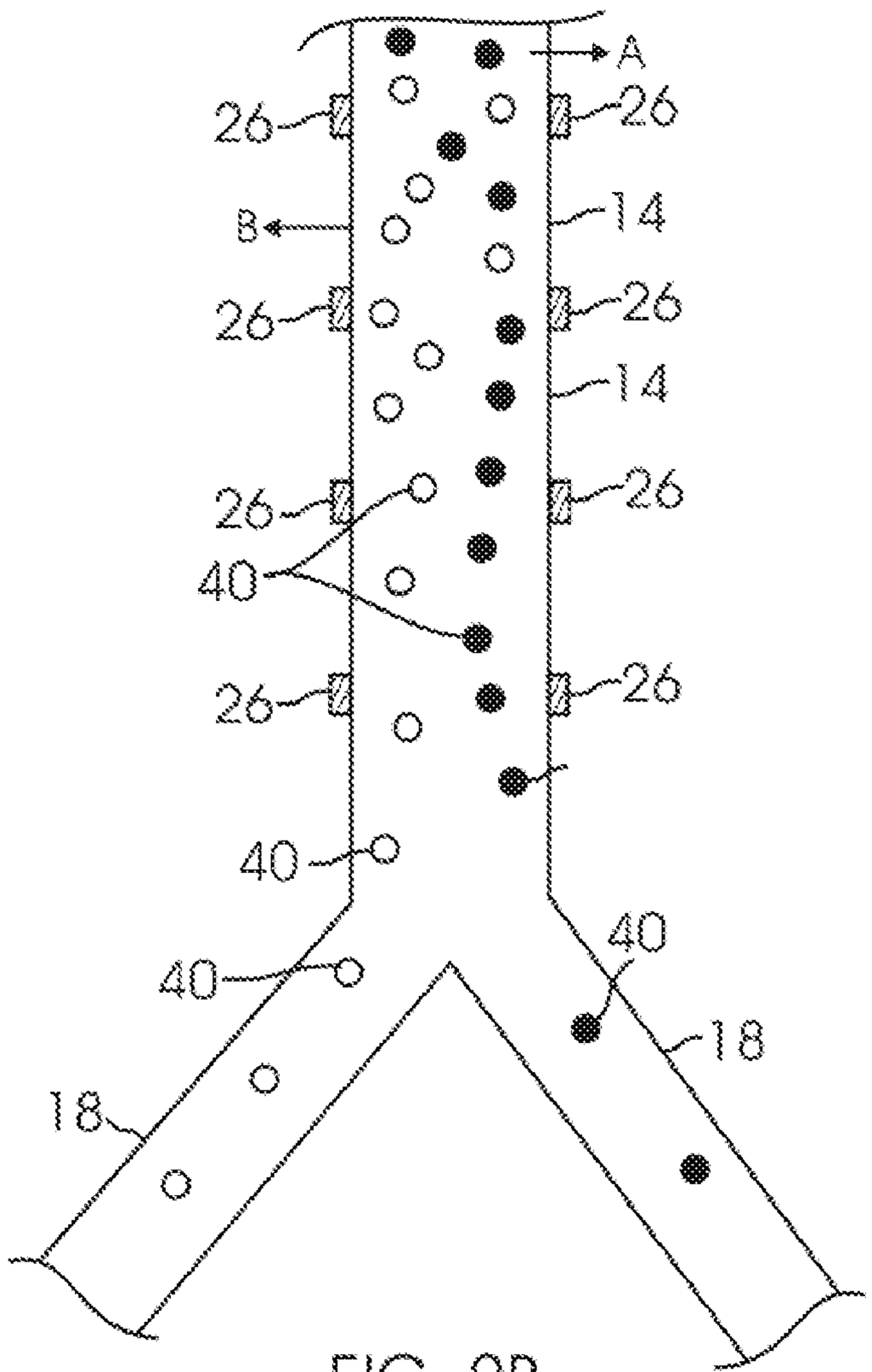

A
B
26
14
40
18

FIG. 2B

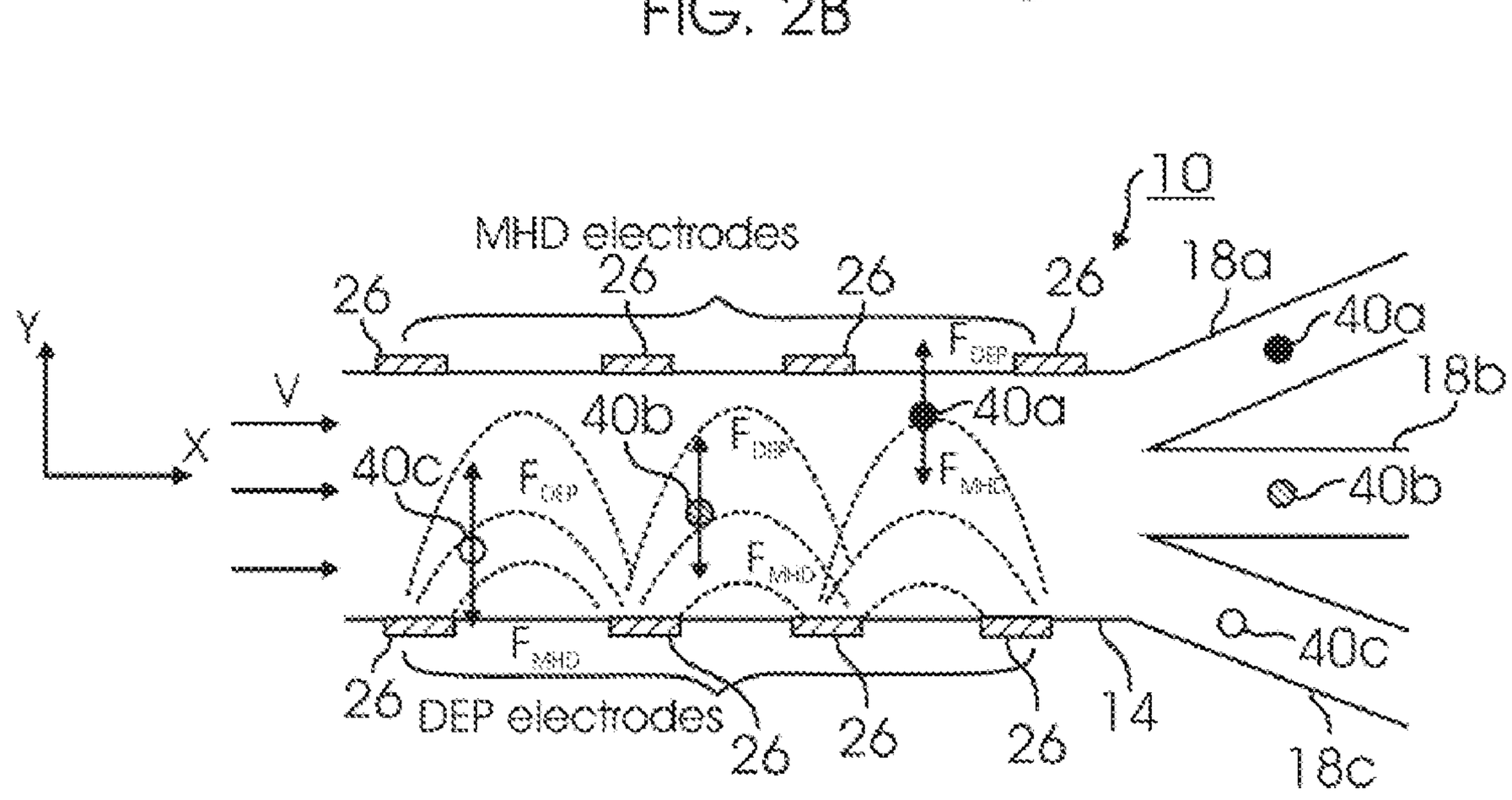

10
MHD electrodes
DEP electrodes
26
18a
18b
18c
40a
40b
40c
14
V
X
Y
F_{DEP}
F_{MHD}

FIG. 3

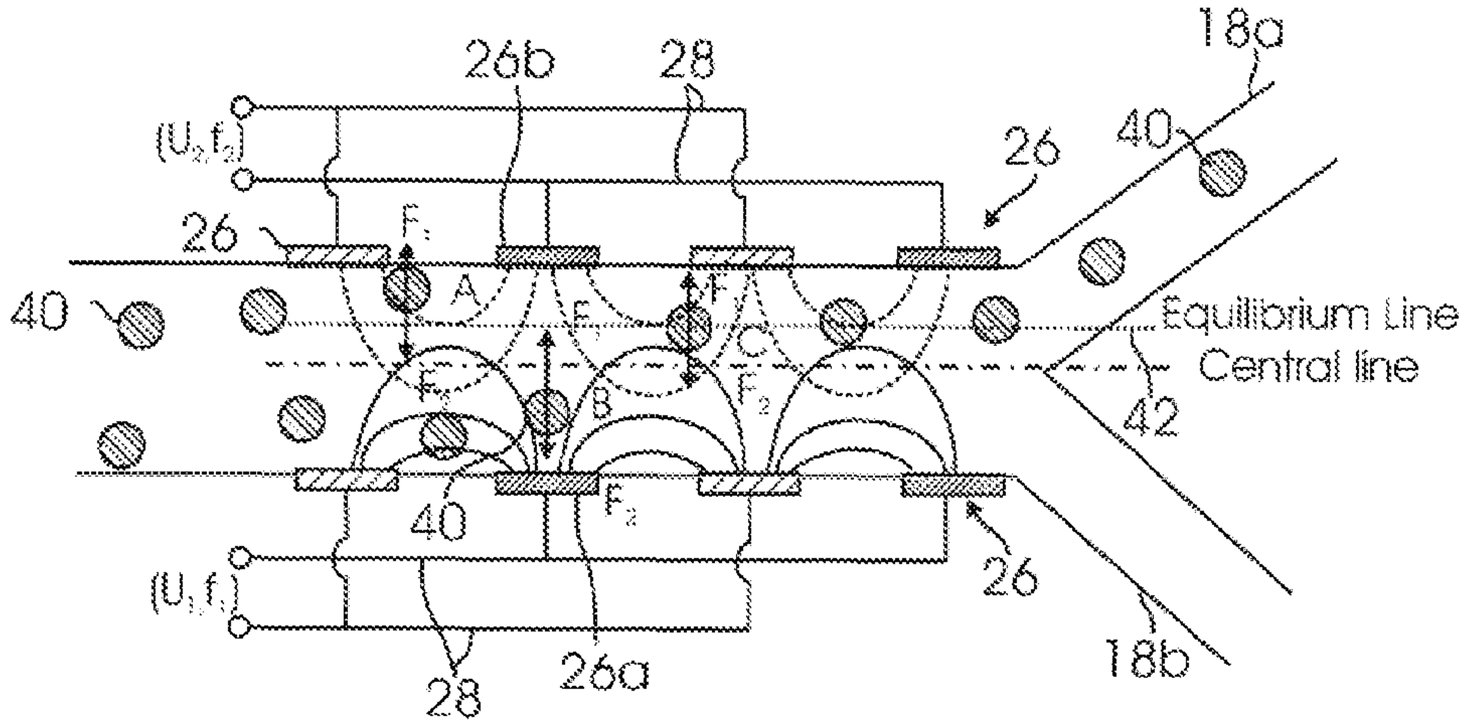

26b
28
18a
40
26
Equillibrium Line
Central line
42
18b
26a

FIG. 4

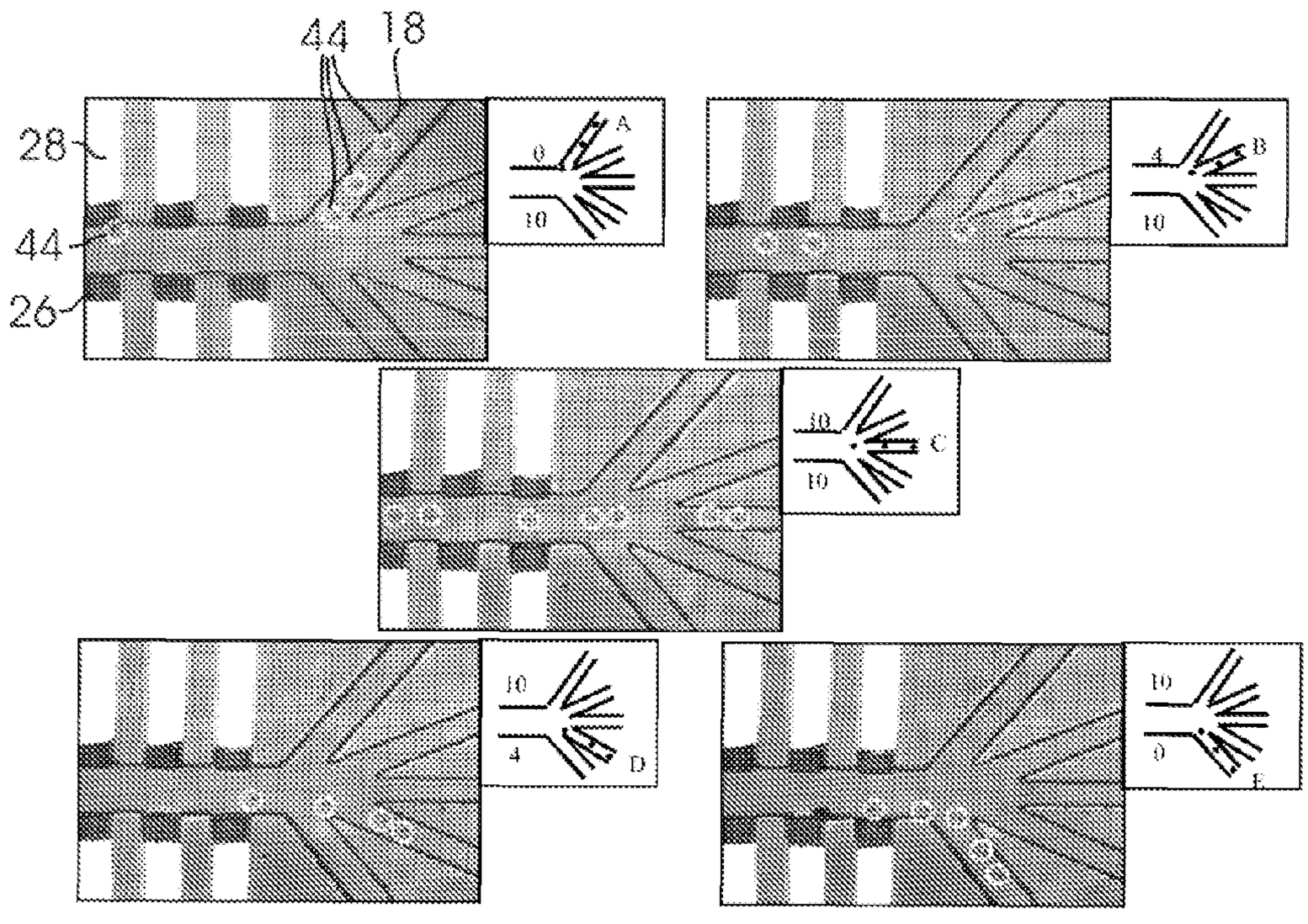

44
18
28
26
A
B
C
D
E

FIG. 5

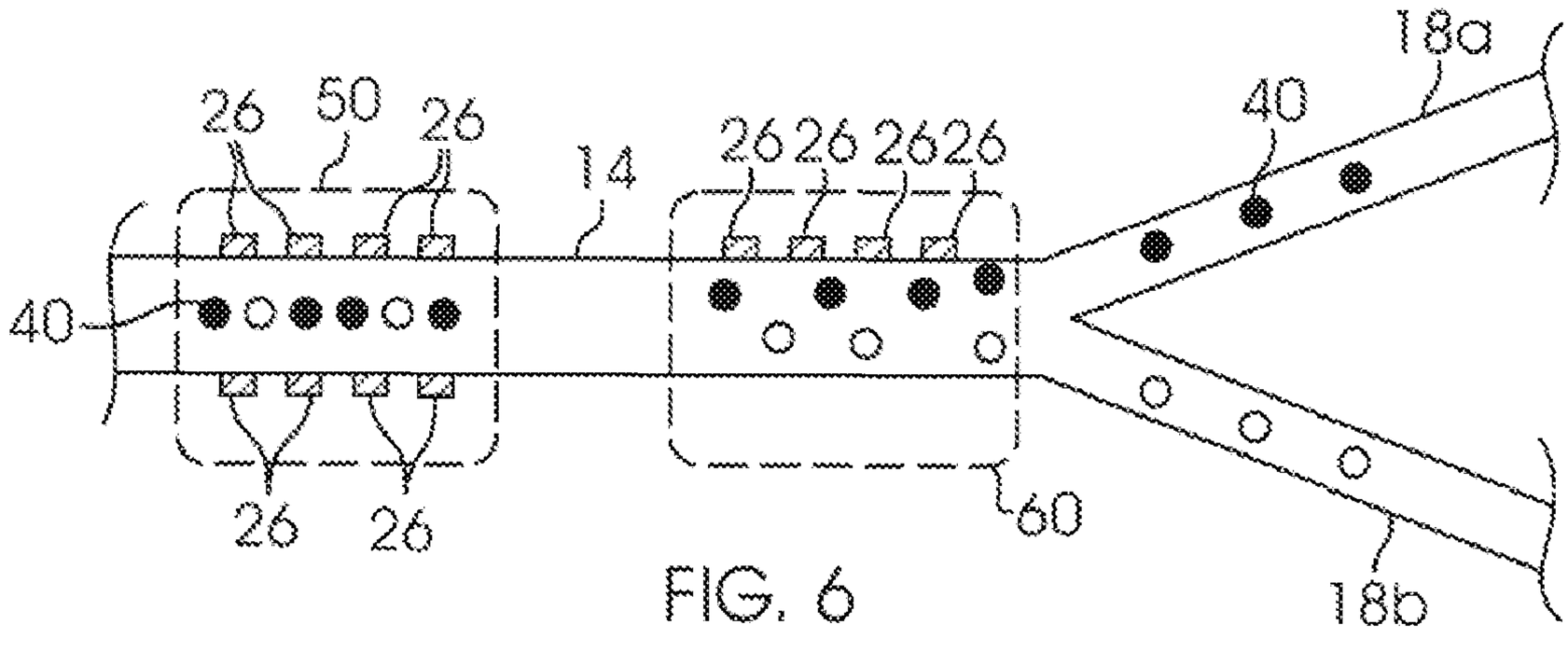


FIG. 6

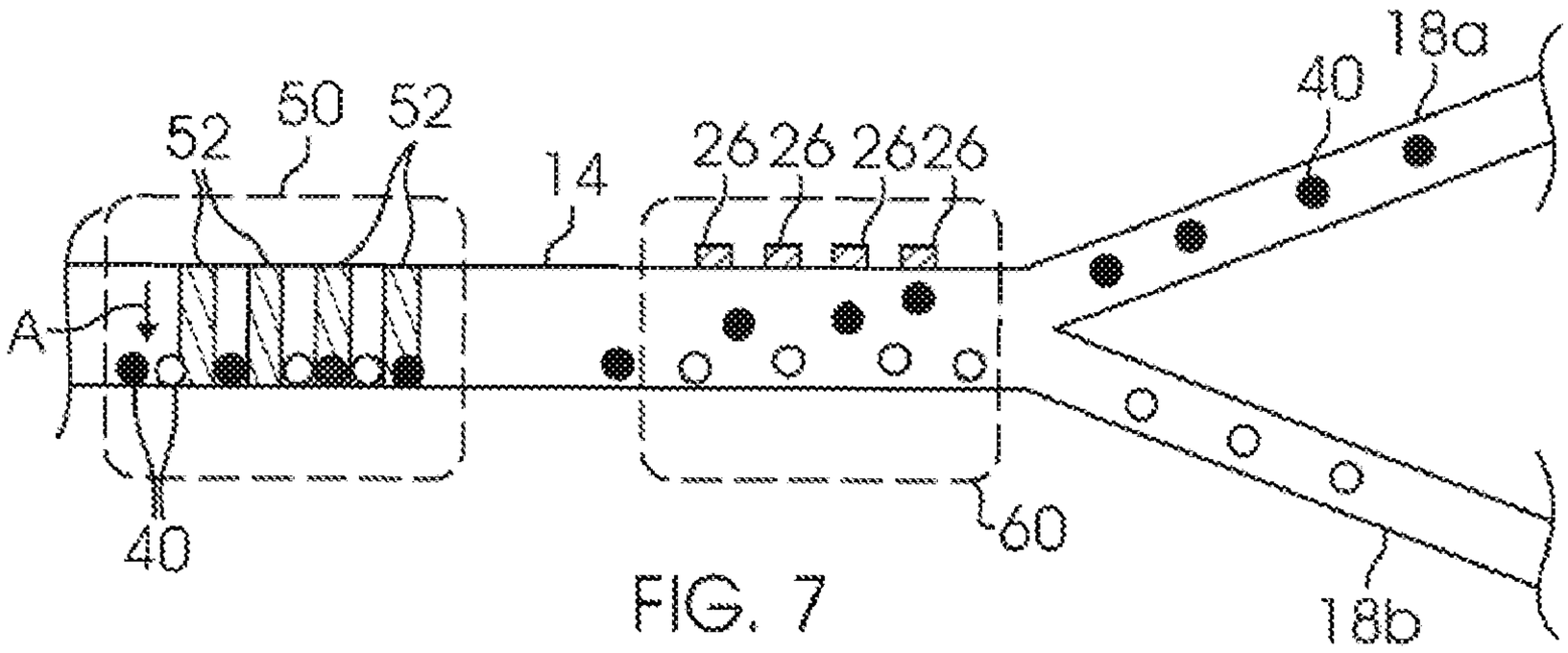


FIG. 7

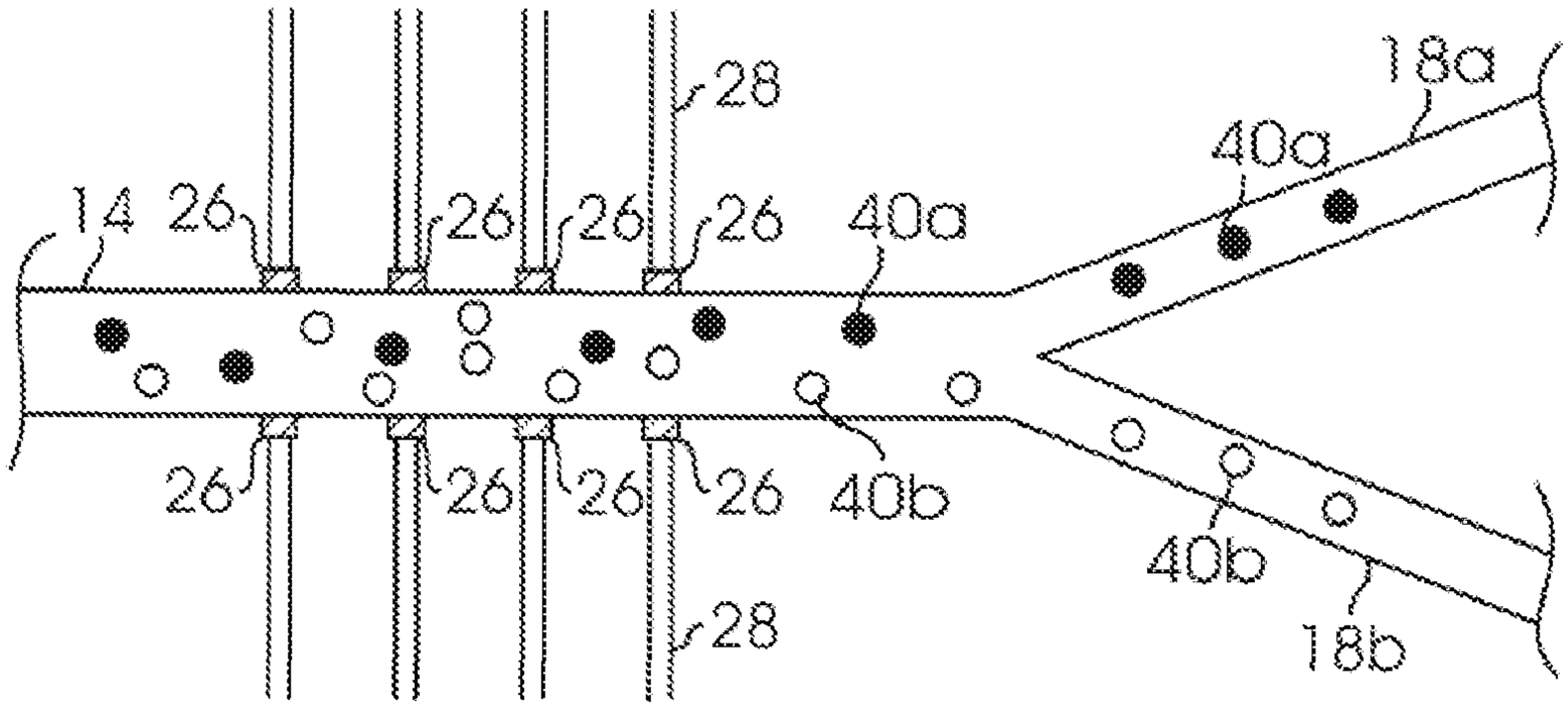


FIG. 8

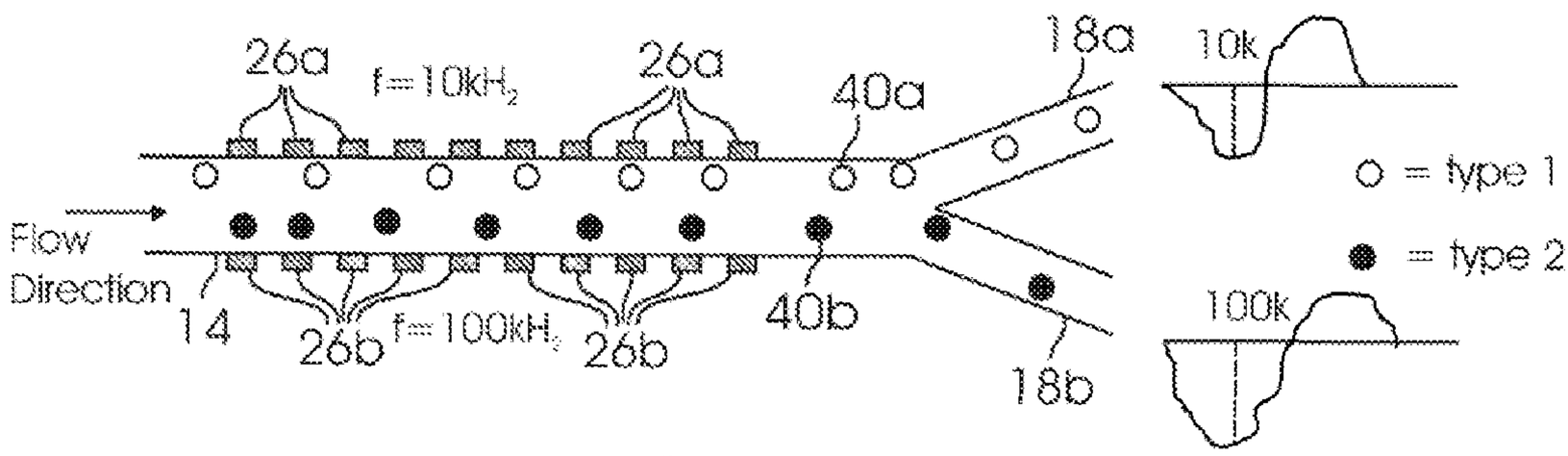


FIG. 9

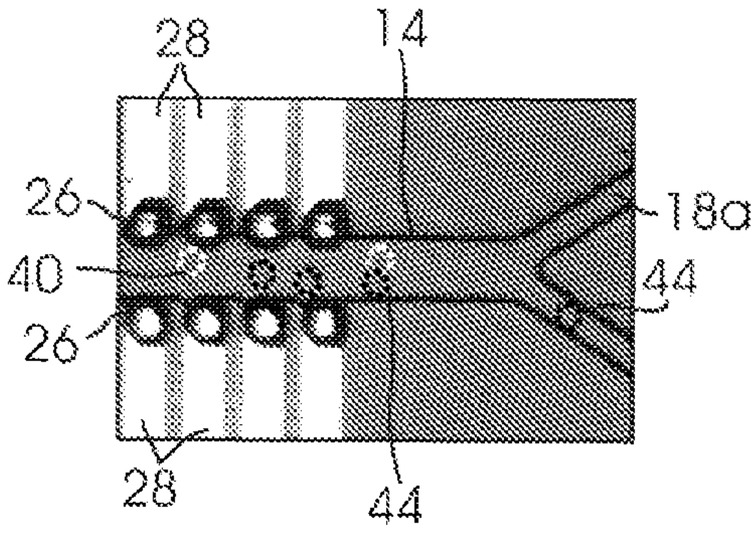


FIG. 10A

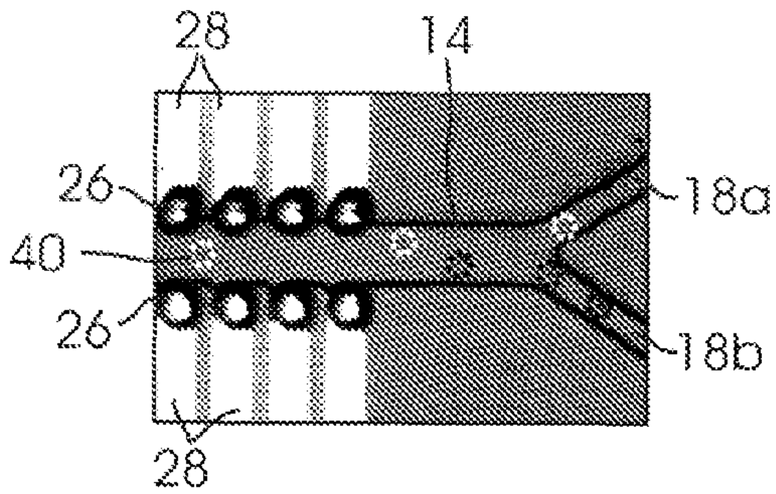


FIG. 10B

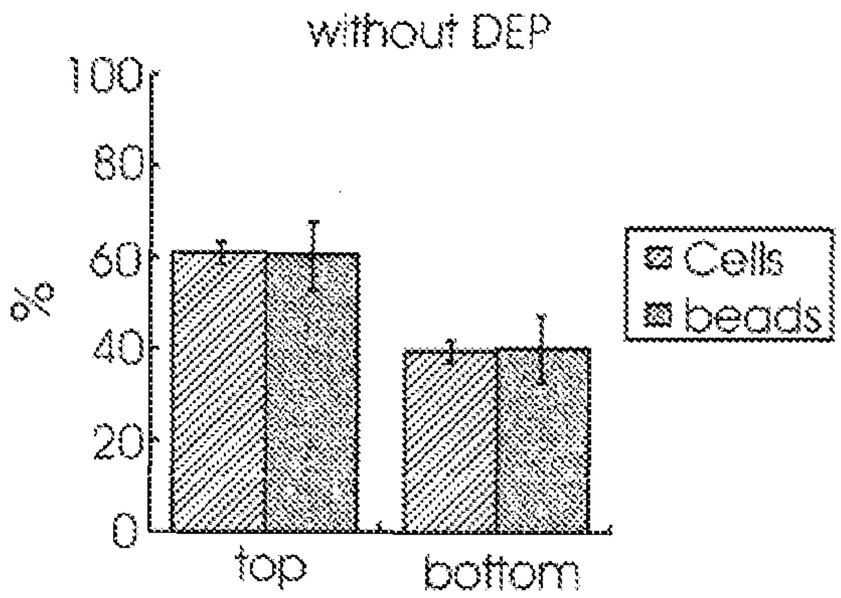


FIG. 11A

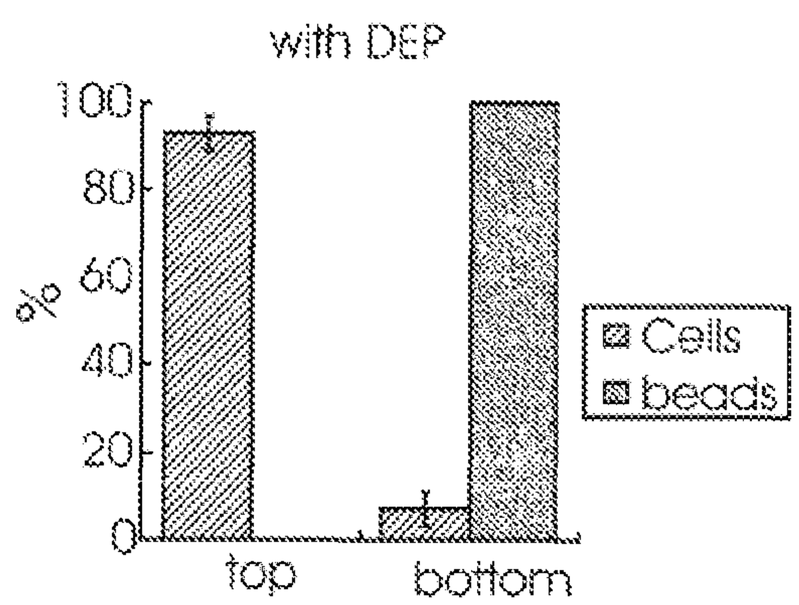


FIG. 11B

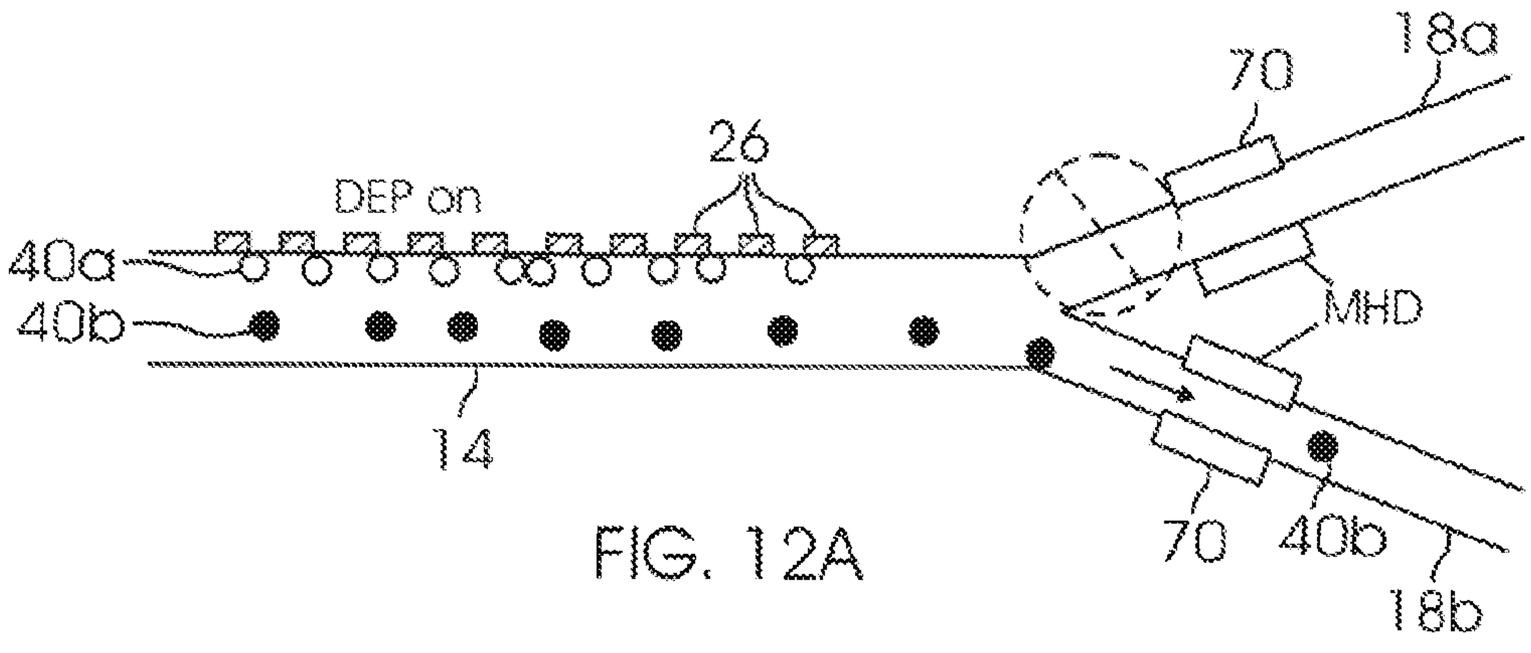


FIG. 12A

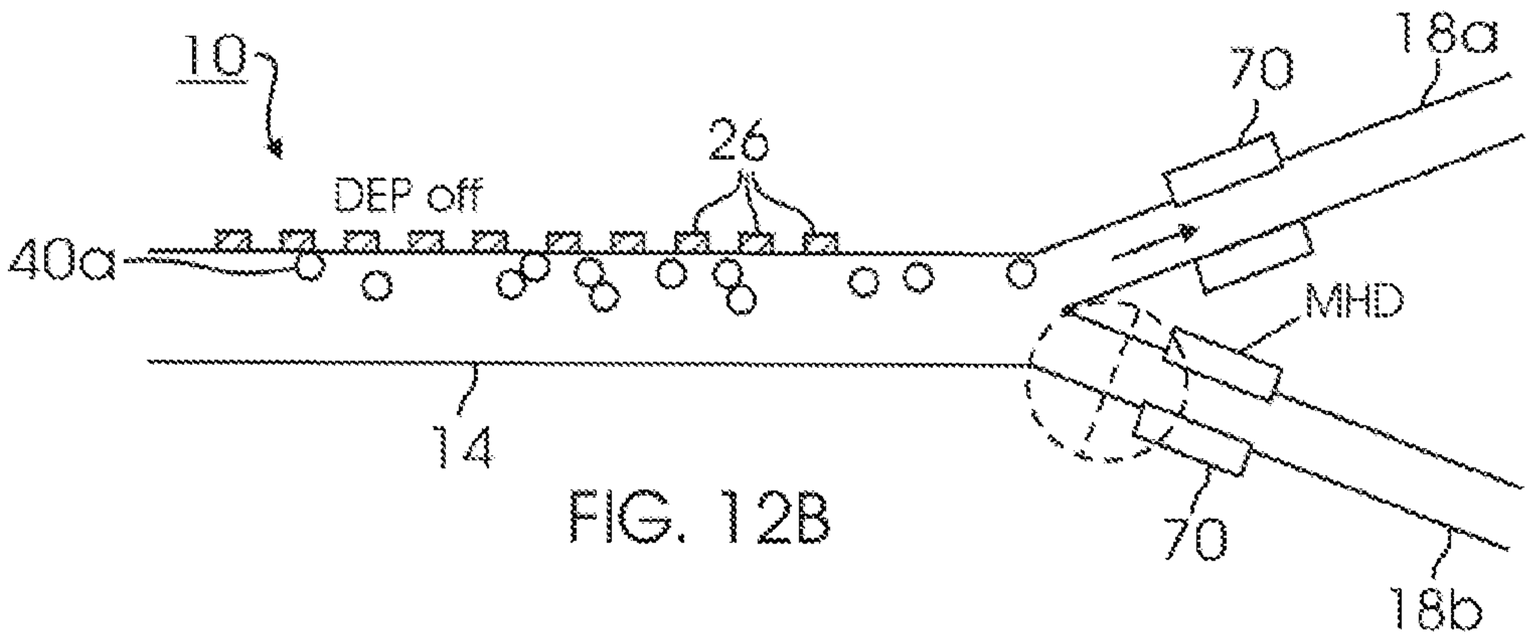


FIG. 12B

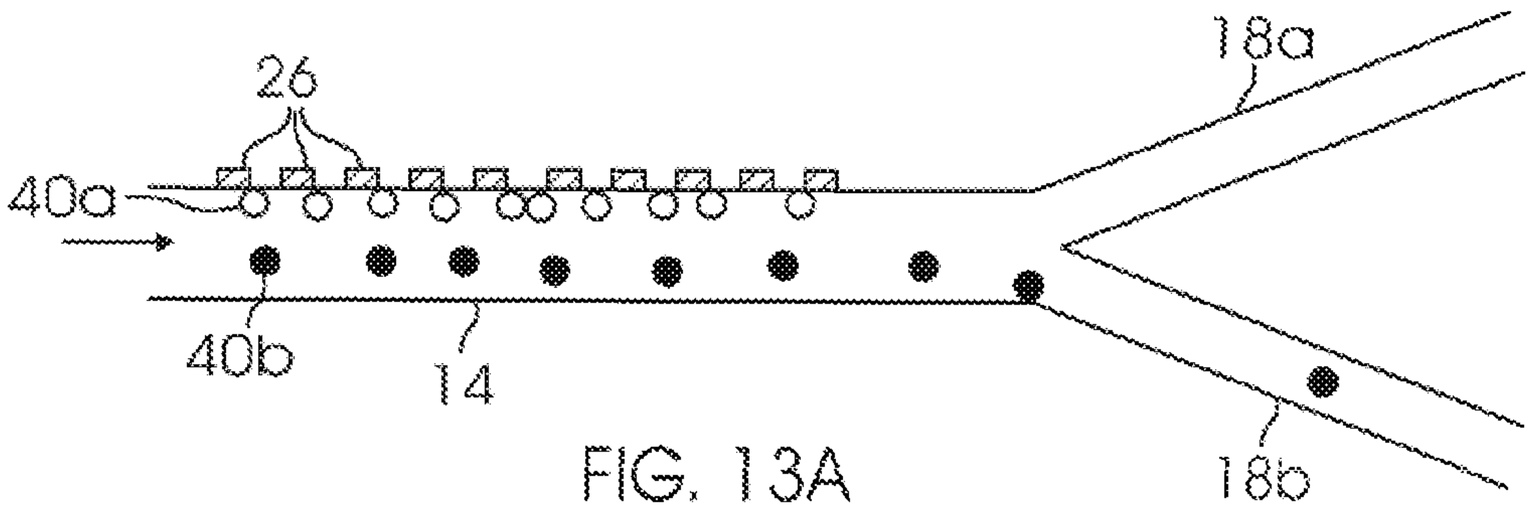


FIG. 13A

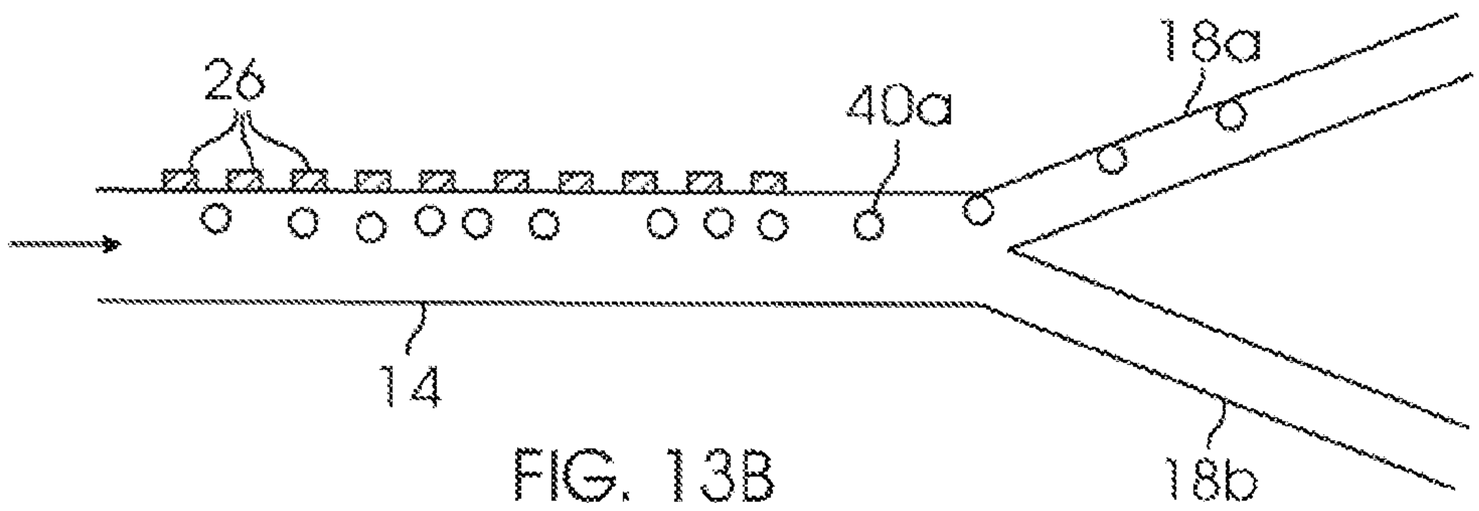


FIG. 13B

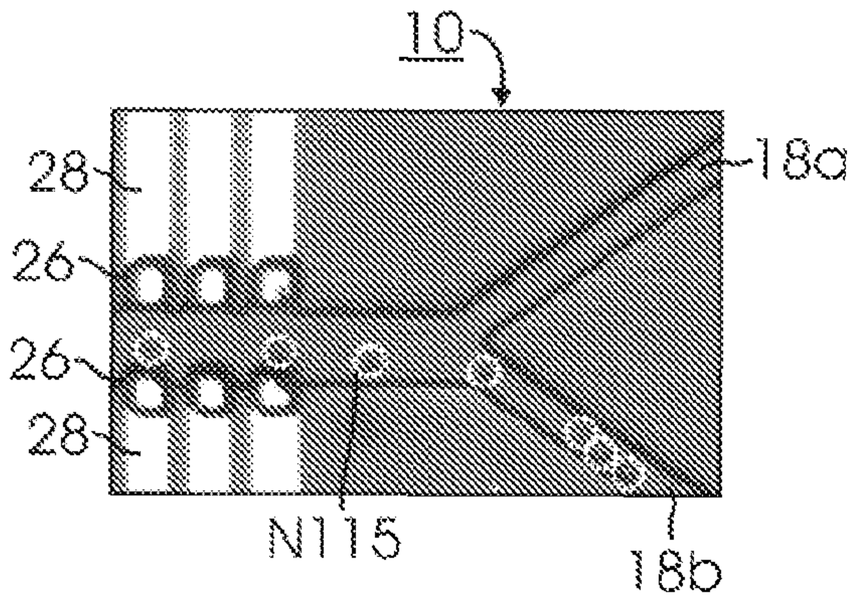


FIG. 14A

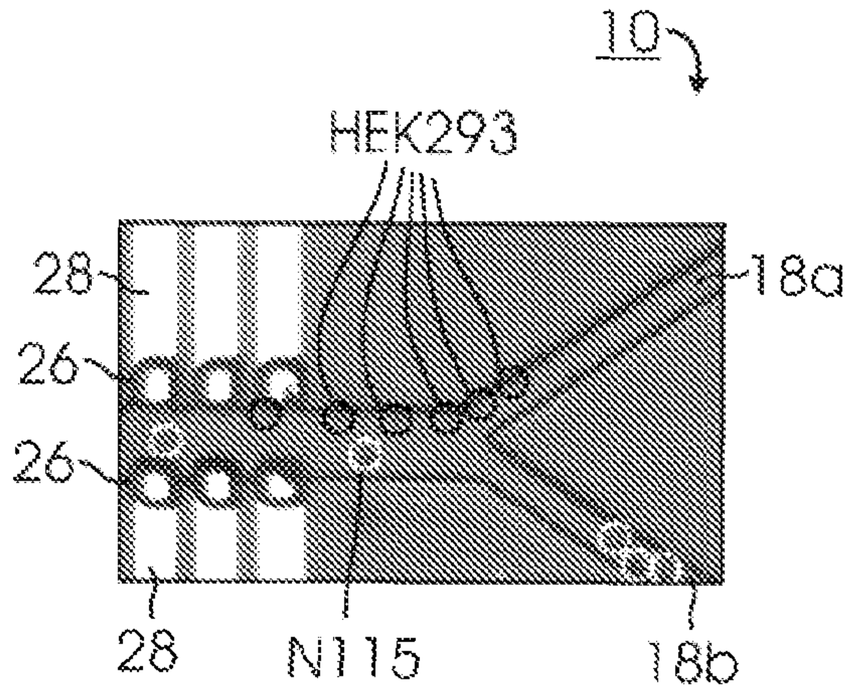


FIG. 14B

MICROFLUIDIC DEVICE FOR CELL AND PARTICLE SEPARATION

REFERENCE TO RELATED APPLICATIONS

This Application claims priority to U.S. Provisional Patent Application No. 60/743,220 filed on Feb. 2, 2006. The '220 application is incorporated by reference as if set forth fully herein. This Application is also a continuation-in-part of U.S. patent application Ser. No. 11/557,060 filed on Nov. 6, 2006, which itself claims priority to U.S. Provisional Patent Application No. 60/734,544 filed on Nov. 7, 2005. The '060 and '544 applications are incorporated by reference as if set forth fully herein.

FIELD OF THE INVENTION

The field of the invention generally relates to devices used to separate and sort particles or cells. More particularly, the invention relates to microfluidic-based devices that separate and/or sort biological materials (e.g., cells or cellular components) or particles.

BACKGROUND OF THE INVENTION

Microfluidic-based systems are becoming widely used in biological and chemical analysis applications. Traditionally, flow cytometry has been used to separate or sort a cell or particle of interest from a heterogeneous population. For example, in conventional flow cytometry, a mixture or cells or particles is hydrodynamically focused using a sheath fluid. The cells or particles, which may be labeled with a fluorescent label or the like, is then interrogated using, for example, a laser or other optical apparatus to identify particular cells or particles of interest within the stream. The cells or particles of interest can then be deflected downstream of the interrogation region into an appropriate collection chamber or the like by using high-voltage electrical plates. For example, the cell or particle contained within the droplet of carrier fluid may be positively or negatively charged which can then be attracted (or repulsed) by the charged electrical plates. This causes movement of the droplets into the proper collection chamber.

More recently, various microfluidic-based sorting schemes have been envisioned to sort cells. For example, Fu et al. discloses a microfabricated fluorescence-activated cell sorter that uses electrokinetic flow to sort bacteria and particles. See Fu et al., A microfabricated fluorescence-activated cell sorter, Nature Biotechnology, 17, 1109-111 (1999). U.S. Pat. No. 6,936,811 discloses a microfluidic sorting device that uses a moving optical gradient to sort particles or cells based on their dielectric properties. Still others have disclosed the use of microfabricated electrodes to separate cells using dielectrophoretic/gravitational field-flow fractionation (DEP/G-FFF). See Yang et al., Cell separation on microfabricated electrodes using dielectrophoretic/gravitational field-flow fractionation, Anal. Chem., 71(5):911-918 (1999). In the DEP/G-FFF method, cells are "levitated" to different heights according to the balance of the DEP and gravitational forces. In still another strategy, cell trapping arrays have been proposed that "trap" cells at dielectrophoretic (DEP) traps. See Heida et al., Dielectrophoretic trapping of dissociated fetal cortical rat neurons, Biomedical Engineering, IEEE Transactions of Biomedical Engineering, Vol. 48, No. 8, August 2001; Taff et al., A Scalable Row/Column-Addressable Dielectrophoretic Cell-Trapping Array, 9th Intl., Conf. on Miniaturized Sys. For Chemistry and Life Sciences, October 2005.

Unfortunately, many of the proposed sorting schemes set forth above have significant limitations. For instance, DEP/G-FFF based devices which rely on the balance between the DEP force and the gravitation force is heavily dependent on the velocity control of the flow since those cells or particles in the middle of the channel are flushed out first because of the parabolic flow profile created within the channel. Also, this method suffers from poor discrimination since the particles/cells located at the sides of the microchannel can be eluted along with the "faster" fraction located within the central region of the channel. In addition, devices using DEP/G-FFF or trapping sort cells or particles temporally (e.g., a time-based approach) making throughput low. Because of this, complicated valves and pumps are needed if this type of separation approach were integrated with other sample preparation steps.

There thus is a need for a device and method that is capable of sorting particles and cells using a spatial approach. Namely, heterogeneous mixtures of cells and/or particles may be automatically directed to downstream channels, branches, or collection chambers without the need for ancillary pumps or valves that control flow patterns. In this regard, the sorting device may be integrated into a microfluidic-based total analysis system that includes other process steps like sample preparation. The device should also permit the sorting of heterogeneous mixtures of cells and/or particles without the need of any fluorescent labels or biomarkers.

SUMMARY

In one embodiment of the invention, a microfluidic separation device includes a microchannel formed in a substrate and being defined at least by a bottom surface, a first side wall, and second side wall. Fluid containing particles or cells is flowed through the microchannel from an upstream end to a downstream end. The downstream end terminates in a plurality of branch channels. A plurality of vertically-oriented electrodes are disposed on the first wall and on the second wall opposite to the first wall. A voltage source is connected to the plurality of opposing electrodes to drive the electrodes. The opposing, vertically-oriented electrodes may be used to focus a heterogeneous population of particles or cells for subsequent downstream separation via additional electrodes placed on one of the side walls. Alternatively, the opposing, vertically-oriented electrodes may be used to spatially separate a heterogeneous population of particles or cells for later collection in one or more of the branch channels.

In another embodiment of the invention, a microfluidic separation device includes a first microchannel formed in a substrate, the first microchannel being defined by a bottom surface, a first wall, and a second wall. The first microchannel includes an upstream end and a downstream end. A focusing region is disposed in the first microchannel and includes a plurality of electrodes disposed on the first wall and another plurality of electrodes disposed on the second wall opposite the plurality of electrodes disposed on the first wall. A voltage source is connected to the plurality of electrodes on the first wall and the second wall. A separation region is disposed in the first microchannel at a location downstream of the focusing region, the separation region comprising a plurality of electrodes disposed on one of the first and second walls of the first microchannel. The device includes a plurality of branch microchannels coupled to a downstream end of the first microchannel.

In another aspect of the invention, a method of sorting particles or cells using a microfluidic device includes providing a microfluidic channel having a bottom surface and two

opposing side walls, the microfluidic channel having a focusing region comprised of a plurality of electrodes disposed on opposing side walls of the microchannel. The microfluidic channel further includes a separation region located downstream of the focusing region and includes a plurality of electrodes on one of the opposing side walls, the microchannel further includes a plurality of branch channels located downstream of the separation region. A heterogeneous population of particles or cells is flowed within the microfluidic channel. The plurality of electrodes in the focusing region are driven to focus or align the heterogeneous population of particles or cells. The plurality of electrodes in the separation region are then driven so as to spatially separate the heterogeneous population of particles or cells. The spatially separated heterogeneous population of particles or cells is then flowed into the plurality of branch channels based, at least in part, on the spatial separation.

In another aspect of the invention, a method of sorting particles or cells using a microfluidic device includes providing a microfluidic channel having a bottom surface and two opposing side walls, the microfluidic channel including a plurality of electrodes disposed on opposing side walls of the microchannel. A plurality of branch channels are connected to main microfluidic channel at a downstream location. A heterogeneous population of particles or cells is flowed within the microfluidic channel. The plurality of electrodes are driven so as to spatially separate the heterogeneous population of particles or cells within the microchannel. The now separated population of particles or cells are then sorted by flow into the plurality of branch channels based, at least in part, on the spatial separation.

In another embodiment of the invention, a method of sorting particles or cells using a microfluidic device includes providing a microfluidic channel having a bottom surface and two opposing side walls, the microfluidic channel including a plurality of DEP electrodes disposed on a side wall of the microchannel. The microchannel further includes a plurality of branch channels located downstream of the plurality of DEP electrodes. A heterogeneous population of particles or cells is flowed within the microfluidic channel. The plurality of DEP electrodes are driven so as to trap a portion of the heterogeneous population of particles or cells at a location within the microchannel that is adjacent to the DEP electrodes. The un-trapped heterogeneous population of particles or cells is then flowed within a first branch channel. The portion of the heterogeneous population of particles or cells that is trapped at the DEP electrodes is then released into the flowing fluid by turning off or de-energizing the DEP electrodes. The released portion of particles or cells is then flowed into a second branch channel.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** illustrates a perspective view of an embodiment of a microfluidic device having a channel with vertical microelectrodes contained therein.

FIG. **2**A illustrates a partial side view of the side wall of a microchannel having a plurality of vertically oriented electrodes.

FIG. **2**B illustrates a top down view of a microfluidic device having a common channel along with two downstream branch channels. Opposing arrays of electrodes are positioned on the side walls of the microchannel.

FIG. **3** illustrates an embodiment of a microfluidic device having MHD and DEP electrodes. Separation and sorting can be accomplished in three downstream branch channels.

FIG. **4** illustrates a DEP-DEP microfluidic device that uses opposing pairs of DEP electrodes to focus or fine tune the equilibrium line of particles flowing through a microchannel.

FIG. **5** illustrates a panel of images illustrating a device of the type shown in FIG. **4** that is able to spatially tune the equilibrium position within the microchannel to selectively switch particles (or cells) into one of five (5) downstream branch channels.

FIG. **6** illustrates one embodiment of a microfluidic device having an upstream focusing region and a downstream separation region.

FIG. **7** illustrates another embodiment of a microfluidic device having an upstream focusing region and a downstream separation region.

FIG. **8** illustrates a microfluidic device in a free flow configuration. The device illustrated uses opposing arrays of vertically oriented DEP electrodes to separate particles or cells.

FIG. **9** schematically illustrates operation of a microfluidic device like that shown in FIG. **8**.

FIGS. **10**A and **10**B illustrate photographic images of cells and microbeads being separated in a microfluidic device like those illustrated in FIGS. **8** and **9**.

FIGS. **11**A and **11**B illustrate graphs showing collection percentage for cells and microbeads with the electrodes in an off state (FIG. **11**A) and on state (FIG. **11**B).

FIGS. **12**A and **12**B illustrate another embodiment of a microfluidic device that traps certain cells or particles of a heterogeneous population using DEP electrodes for subsequent separation and sorting.

FIGS. **13**A and **13**B illustrate another embodiment of a microfluidic device that traps certain cells or particles of a heterogeneous population for subsequent separation and sorting.

FIGS. **14**A and **14**B illustrate photographic images of a trapping microfluidic device that is used to separate and sort N115 cells from HEK293 cells.

DETAILED DESCRIPTION OF THE INVENTION

FIG. **1** illustrates a microfluidic device **10** according to one embodiment of the invention. The microfluidic device **10** includes a substrate **12** onto which the device **10** is formed. The substrate **12** may include a relatively inert material such as silicon, glass, polycarbonate, or a plastic-based material. The substrate **12** should be amenable to depositing the electrodes and electrical lines or traces used to drive the electrodes (discussed in more detail below).

A main or common microchannel **14** is formed in a polymer-based material **16** that overlays the substrate **12**. The polymer-based material **16** may include a photoresist such as, for instance, SU-8. As seen in FIG. **1**, the microchannel **14** has a length that includes an upstream region **14***a*, and a downstream region **14***b*. The terms upstream and downstream are indicative of the direction of flow of fluid and particles or cells within the microchannel **14** during operation. During operation, fluid flow (and hence flow of particles or cells) goes from the upstream region **14***a* toward the downstream region **14***b*.

As seen in FIG. **1**, the main or common microchannel **14** includes a plurality of downstream branch channels **18** that are joined to the common microchannel **14** at the downstream region **14***b* at a junction **20**. In the embodiment of FIG. **1**, two branch channels **18** are illustrated but the device **10** may include any number of branch channels **18** greater than two (2). Also, as seen in FIG. **1**, the branch channels **18** terminate in two collection chambers **22** which, in certain embodiments, may be used to collect the sorted particles or cells. It

should be understood, however, that the branch channels **18** may be coupled to other microfluidic channels, mixing regions, reaction chambers, and the like, which can be used for various post-sorting operations. In this regard, the device **10** is particularly well suited for integrated lab-on-a-chip devices or systems which integrate numerous processes into a single device.

FIG. **1** also illustrates an inlet chamber **24** that is coupled to the upstream region **14***a* of the main microfluidic channel **14**. A heterogeneous population of cells or particles may be loaded into the device **10** via the inlet chamber **24**. The population of cells or particles would, of course, be loaded or carried by a carrier fluid. The carrier fluid is typically a biologically-compatible fluid such as, for example, a water-based isotonic solution. In the case of particles, however, it may be possible to use one or more organic-based carrier fluids. The inlet chamber **24** may, alternatively, be coupled to fluid source via tubing or conduit to a pump or the like that loads and transports cells or particles into the device **10**. In still another alternative, various microfluidic channels, chambers, or the like (not shown) may be coupled to the upstream portion **14***a* of the main channel **14**. For example, various sample preparation processes may take place upstream of the main channel **14** in one or more microfluidic features that are contained on an integrated device **10**.

Still referring to FIG. **1**, a plurality of opposing, vertically-oriented electrodes **26** are disposed on opposite sides of the side walls of the microchannel **14**. The vertically-oriented electrodes **26** may be formed from an electrically-conductive material such as, for instance, platinum, gold, carbon, or other biologically inert material. As seen in FIG. **1**, the electrodes **26** generally are formed in pairs with adjacent electrodes **26** being of opposite polarity (as seen in FIG. **2**B). FIG. **1** illustrates two pairs on either side of the microchannel **14** (i.e., a total of four electrodes **26** on each side) but there may be more depending on the particular application.

Each electrode **26** is coupled to respective electrical traces or lines **28**. The electrical traces or lines **28** may be formed from an electrically-conductive material such as, for instance, gold, platinum or the like. As seen in FIG. **1**, each electrical trace or line **28** may terminate in an electrical contact or pad **30**. The electrical contact **30** for each electrode **26** may then be connected to a voltage source **32** via leads or wires **34**. As seen in FIG. **1**, separate voltage sources **32** (V_1 and V_2) may be connected to the electrodes **26** on the opposing side walls of the microchannel **14**. In the case of dielectrophoresis (DEP) electrodes **26**, the voltage source **32** preferably has the ability to adjust the applied frequency (AC mode) as well as the voltage (e.g., amplitude). For example, a dual channel function generator like the Tektronix AFG320 may be used to input signals to the opposing electrode arrays of electrodes **26** on either side of the microchannel **14**. The particulars of the forces generated by the electrodes **26** is discussed more detail herein.

The nature of the electrodes **26** along the opposing side walls of the microchannel **14** may vary. For example, in one embodiment (e.g., FIG. **4**) the opposing plurality of electrodes are all DEP electrodes. In particular, DEP electrodes induce forces on the cells or particles that is generally perpendicular to the direction of flow within the microchannel **14**. The forces are a function of, among other things, the dielectric constant of the particle or cell. The forces may be attractive or repulsive. In another embodiment, the opposing plurality of electrodes **26** along the walls of the microchannel **14** may be magneto-hydrodynamic electrodes (MHD). MHD electrodes are typically driven by AC current although in some applications DC current may be applied. MHD electrodes typically operate by application of AC electrical current and a perpendicular sinusoidal AC magnetic field that pass through an electrolytic solution. This produces a Lorentz force, which in embodiments described herein, is generally perpendicular to the direction of fluid flow (i.e., from one side wall to the other).

In the embodiments described herein, MHD electrodes **26** may be oriented vertically along the walls of the microchannel **14**, for example, as shown in FIG. **3**. Alternatively, the MHD electrodes **26** may be oriented perpendicular to the direction of flow within the microchannel **14**, as is shown in FIG. **7**. MHD electrodes **26** produce a substantially uniform force on the carrier fluid that contains the particles or cells. MHD electrodes **26** are thus well suited for pumping applications as well as focusing or alignment procedures as discussed in more detail below.

The electrodes **26** may be arranged within the microchannel **14** in any number of configurations that are suitable for the particular application of interest. For example, the electrodes **26** may interdigitated on opposing side walls of the microchannel **14**. The electrodes **26** may be symmetric or unsymmetric depending on the desired field effect (e.g., DEP or MHD field). The electrodes **16** may be smooth or have sharp edges. In addition, in some embodiments, such as that disclosed later in FIG. **7** may include MHD electrodes **26** arranged flat on the bottom surface of the microchannel **14**. In addition, DEP electrodes **26** may be arranged in a flat or flush orientation with the side walls of the microchannel **14**.

Still referring to FIG. **1**, an optional cap or cover **36** is positioned over the polymer-based material **16** in which the microchannel **14** is formed. Preferably, the polymer-based material **16** is flexible so that a good seal can be formed between the cover **36** and the underlying material **16**. Of course, it may be possible to use a rigid or semi-rigid material for the cap **36**. For example, glass or plastic may be used to form the cap **36**.

The microfluidic device **10** may formed using a combination of photolithographic, metal deposition, and electroplating techniques. For example, the substrate **12** may be coated with an adhesion layer of photoresist (SU-8) followed by deposition and patterning of thin metal layers for the electroplating of the vertically oriented electrodes **26**. The electrodes **26** may be electroplated through a SU-8 mold layer which is then removed. The SU-8 channel layer **16** can be coated and patterned with alignment of the microchannel **14** and the electrodes **26**. The alignment of the electrodes **26** and the side walls of the microchannel **14** ensures that the electrodes **26** can be exposed to the fluid contained within the microchannel **14**. A flexible PDMS sheet coated with a thin layer of SU-8 may be used as the cap **36** to seal the microchannel **14**. Further details on the process of forming vertically oriented electrodes **26** on the side walls of the microchannel **14** may be found in U.S. patent application Ser. No. 11/557,060 which is incorporated by reference as if set forth fully herein.

The opposing vertical electrodes **26** may be located along the full height or depth of the side walls forming the microchannel **14** so as to avoid any dead electrical field zones. This is illustrated in FIG. **2**A, which shows three such electrodes **26** traversing the entire height of the microchannel **14**. FIG. **2**B illustrates a top-down view of a microchannel **14** with two branch channels **18**. Also shown are a heterogeneous population of cells **40**. The population illustrated in FIG. **2**B has two types of cells (represented by solid circles and open circles). It should be understood that the cells **40** could also be particles and still work in accordance with the invention described herein.

In this embodiment, one type of cells **40** (i.e., solid circles) experience a force in the direction of arrow A while the other type of cell **40** (open circles) experience a force B in the direction of arrow B. In this embodiment, both sets of opposing electrodes **26** are DEP electrodes. As the heterogeneous population of cells **40** passes through the region of the microchannel **14** that contains the electrodes **26**, the cells are spatially separated along the width of the microchannel **14**. Specifically, the cells **40** represented by the solid circles move toward and ultimately into the branch channel **18** on the right while the cells represented by the open circles move toward and ultimately into the branch channel **18** on the left. It should be understood that the spatial separation of the cells **40** determines what branch channel **18** a particular cell **40** will go into.

FIG. **3** illustrates a top view of an embodiment of a device **10** that includes a microchannel **14** having a plurality of vertically oriented, MHD electrodes **26** positioned along one side of the wall of the microchannel **14**. The other, opposing side wall of the microchannel **14** has a plurality of vertically oriented, DEP electrodes **26**. Three types of cells **40***a*, **40***b*, **40***c* are shown being directed into three downstream branch channels **18***a*, **18***b*, **18***c*. Fluid flow is in the direction of the arrows V in FIG. **3**. FIG. **3** illustrates the electrical field from the DEP electrodes **26**. Also shown is the force balance diagram for the DEP force (F_{DEP}) and the MHD force (F_{MHD}). The MHD force is generally perpendicular to the direction of flow (V) within the microchannel **14** and tends to move the cells **40***a*, **40***b*, **40***c* toward the side of the microchannel **14** containing the DEP electrodes **26**. The MHD force is typically a substantially uniform force as it generally acts upon the fluid carrying the cells **40***a*, **40***b*, **40***c*.

In contrast, the DEP force, while also being directed perpendicular to the direction of fluid flow (V), tends to move the cells **40***a*, **40***b*, **40***c* in the opposite direction, toward the side wall of the microchannel **14** having the MHD electrodes **26**. The DEP force is non-uniform and affects the different types of cells **40***a*, **40***b*, **40***c* differently. For example, the cell **40***a* experiences a large DEP force, the pushes the cell **40***a* towards the top of the microchannel **14**. Because of this, the cell **40***a* is transferred into the top branch channel **18***a*. The cell **40***b* experiences a medium DEP force and pushes the cell **40***b* generally in the middle of the microchannel **14** as seen in FIG. **3**. The cell **40***b* then flows downstream and into the middle branch channel **18***b*. Finally, the cell **40***c* experiences a light DEP force. As a result, the cell **40***c* generally stays close to the lower region of the microchannel **14** and then flows into the lower branch channel **18***c*.

FIG. **4** illustrates yet another embodiment wherein vertically oriented DEP electrodes **26** are positioned on both side walls of the microchannel **14**. FIG. **4** further illustrates the electrical lines or traces **28** that connect the electrodes **26**. As seen in FIG. **4**, the electrodes **26** alternate in polarity along the length of the side wall of the microchannel **14** (e.g., arranged in an interdigitated manner). In the configuration of FIG. **4**, the DEP force created by the DEP electrodes **26** on one side wall can be used to counteract and balance against the DEP force created by the DEP electrodes **26** on the opposing side wall of the microchannel **14**.

FIG. **4** further illustrates the force balance of particles between the two sets of side wall electrode arrays. A signal (U_1, f_1) is applied to the bottom array of electrodes **26**, generating an upwards negative DEP force F_1 on the particles. In FIG. **4**, U represents a voltage while f represents an applied frequency. A second signal (U_2, f_2) is applied on the top electrodes **26** to generate a counter force F_2 to the force generated by the first (i.e., top) set of electrodes **26**. By varying the magnitude and frequencies of the voltages, the forces (F_1, F_2) from these two sets of electrodes **26** can balance each other, and the cells **40** (or particles) can therefore be spatially positioned at an equilibrium point **42**. The equilibrium point **42**, in which the two opposing forces F_1, F_2 equal each other, is unique for a particular cell type **40** and can be used as a basis for downstream sorting as described herein. For example, a cancerous cell **40** may have an equilibrium point **42** at a spatial location nearer to the upper electrodes **26** while a non-cancerous or normal cell **26** may have an equilibrium point **42** at a spatial location nearer to the lower electrodes **26**.

Still referring to FIG. **4**, when a cell **40** is close to electrode **26***a*, like the one at point B, the DEP force F_1 from electrode **26***a* is larger than the DEP force F_2 from electrode **26***b*, the cell will be pushed upwards (because of the larger repulsive force from electrode **26***a*). With the movement away from the bottom electrode **26**, the DEP force F_1 decreases and F_2 increases until it reaches an equilibrium position **42** where F_1 is equal to F_2 at point C. The line that passes through point C along the direction of the microchannel **14** is the equilibrium point **42** (or line) for the cells **40** at the applied driving frequency and amplitude. The two DEP forces (F_1, F_2) can be adjusted by changing the voltage U or the frequency f, so that the equilibrium line **42** can be tuned to any location along the width of the microchannel **14**. For example, tuning can be used to displace the equilibrium line **42** above the centerline of the microchannel **14** as is shown in FIG. **4**. In this configuration, the cells **40**, which are moving in a continuously flowing carrier fluid, are moved to the upper branch channel **18***a*. Alternatively, the equilibrium line **42** may be tuned to fall bellow the centerline and thereby cause the cells **40** to pass into the lower branch channel **18***b*.

This embodiment is suited for forming devices **10** that acts as a microfluidic switch. Because of the effect of the coupled DEP forces, objects of interest such as cells **40** (or particles) can be positioned at any equilibrium position in the microchannel **14**. The device **10** thus permits spatial tuning in the lateral direction within the microchannel **14**. The embodiment is particularly well-suited for switching applications that having multiple outlets or branch channels **18**. Only two electrical signal inputs are required to switch the cells **40** or particles to multiple channels. This type of design may be integrated together with other cell separation techniques such as field flow fractionation to switch multiple types of cells **40** at different times in a continuous manner. This design is also very compatible with other microfluidic sample preparation steps. Multiple types of objects (e.g., cells or particles) can be serially switched to different branch channels **18** and processed in parallel. The ability to continuously flow samples through a channel has great potential for high throughput microfluidic flow cytometry.

FIG. **5** illustrates a panel of images illustrating a device **10** of the type shown in FIG. **4** that is able to spatially tune the equilibrium position within the microchannel **14** to selectively switch particles **44** into one of five (5) downstream branch channels **18**. Experiments were conducted using polystyrene microbeads **44** that were introduced into a microchannel **14** having four DEP electrodes **26** on opposing side walls of the microchannel **14**. The downstream portion of the microchannel **14** terminated in five (5) branch channels **18**.

The carrier fluid for the polystyrene microbeads **44** was an isotonic medium (8.5% sucrose (w/v), 0.3% dextrose (w/v)) dissolved in double deionized water (DDI). The conductivity was adjusted to 0.1 mS/cm with 1640 RPMI used as the DEP buffer solution (using ThermoOrion conductivity/pH meter). The polystyrene microbeads **44** were 6 micron microbeads obtained from a flow cytometry calibration kit (Cat #F13838) (available from Molecular Probe, Inc. of Oregon) and were

diluted into the buffer solution after sonication. The solution was then introduced into the microchannel **14** with a controlled flow rate by a PicoPlus (Harvard Apparatus, MA) syringe pump. A dual-channel function generator (Tektronix AFG320) was used to input two separate signals to the two arrays of electrodes **26** (voltage and frequency). The trajectory of the particle motion was recorded using a CCD camera (Photron FASTCAM). The panel of images along with a corresponding graphic representation is shown in FIG. **5**. The microbeads **44** appeared to have negative DEP properties in the selected medium with different applied frequencies.

Still referring to FIG. **5**, the microbeads **44** were focused or tuned to a variety of spatial locations within the microchannel **14**. The tuning was accomplished by changing the either the voltage or the applied frequency on the electrodes **26**. As seen in FIG. **5**, there are five (5) outlet channels **18**. Since the K factor for the microbeads **44** is almost constant at the selected medium conductivity with respect to frequency, switching by voltage difference applied on the two sets of opposing, vertically oriented electrodes **26** was demonstrated. When the microbeads **44** are flowing in the microchannel **14**, their trajectory can be adjusted to be away from the centerline of flow (e.g., streamline). As seen in FIG. **5**, switching can be extended to channels with multiple outlets. DEP switching thus redirects the objects from the stream line and aligns them towards different outlets or branch channels **18**. The top and bottom electrode arrays are applied with same frequency but different amplitudes of voltage to generate different DEP forces. Because the microbeads **44** experienced a negative DEP force, the force from the electrodes **26** with the higher voltage amplitude will be stronger and therefore will deflect the microbeads **44** towards the opposing electrodes **26** driven at the lower amplitude. The equilibrium point(s) **42** for the microbeads **44** can be tuned to any spatial location the width of the microchannel **14**. Because of this it is relatively straight forward to expand the switching to multiple outlets or branches **18**. While FIG. **5** shows the switching of microbeads **44** into five (5) outlets or branch channels **18** this number could be increased or decreased as needed. In this experiment the microbeads **44** were switched to five different outlet channels sequentially.

As seen in the upper left image of FIG. **5**, a voltage of 0 was applied to the top DEP electrodes **26** while a voltage of 10 was applied to the bottom electrodes **26**. This caused the microbeads to pass into branch channel A. In the upper right image of FIG. **5**, a voltage of 4 was applied to the top DEP electrodes **26** while a voltage of 10 was applied to the bottom electrodes **26**. This caused the microbeads to pass into branch channel B. In the upper middle image of FIG. **5**, a voltage of 10 was applied to the top DEP electrodes **26** while a voltage of 10 was applied to the bottom electrodes **26**. The DEP forces thus being balanced, this caused the microbeads to pass into branch channel C. In the lower left image of FIG. **5**, a voltage of 10 was applied to the top DEP electrodes **26** while a voltage of 4 was applied to the bottom electrodes **26**. This caused the microbeads to pass into branch channel D. In the lower right image of FIG. **5**, a voltage of 10 was applied to the top DEP electrodes **26** while a voltage of 0 was applied to the bottom electrodes **26**. This caused the microbeads to pass into branch channel E.

FIG. **6** illustrates another embodiment of a device **10**. In this embodiment, the microchannel **14** includes an upstream focusing region **50** and a downstream separation region **60**. The main or common microchannel **14** terminates into a plurality of downstream branches **18** as in the other embodiments described herein. The focusing region **50** is used to focus or align cells **40** or particles **44** to a common, starting location from which separation will occur at a downstream location. In the embodiment of FIG. **6**, the focusing region **50** includes opposing arrays of vertically oriented electrodes **26** as described herein. For example, the opposing arrays of electrodes **26** may include DEP electrodes **26** which impart forces in opposing directions on each cell **40** or particle **44**. In FIG. **6**, two types of cells **40** are aligned or focused in the middle of the microchannel **14** by opposing pairs of DEP electrodes **26**.

Once the cells **40** are aligned at a starting location, the cells **40** progress downstream to a separation region **60**. In the embodiment of FIG. **6**, the separation region comprises an array of vertically oriented DEP electrodes **26** positioned on one side wall of the microchannel **14**. Because each cell **40** experiences a different DEP force from the activated electrodes **26** in the separation region **60** the particles become spatially separated in the lateral (i.e., width) direction. As seen in FIG. **6**, the solid cells **40** experience very little DEP force and, as a result, fluid flow carries these cells **40** into the upper branch channel **18***a*. In contrast, the other type of cells **40** (represented by open circles) experiences a larger repulsive force from the active DEP electrodes **26** in the separation region **60**. Because of this, these cells **40** are pushed toward the opposing side wall which, in this embodiment, does not have any electrodes **26**. The flow of fluid then carries these cells **40** into the lower branch channel **18***b*.

FIG. **7** illustrates another alternative embodiment of a device **10** using both a focusing region **50** and a downstream separation region **60**. In this embodiment, however, a plurality of MHD electrodes **52** are located on a bottom surface of the microchannel **14**. The MHD electrodes **52** are generally oriented transverse to the direction of flow within the microchannel **14**. The MHD electrodes **52**, when driven by a voltage source (not shown), produces a force on the carrying fluid in the direction of arrow A. This causes the cells **40** (or particles **44**) to move toward one side of the microchannel **14** as is illustrated in FIG. **7**. Here, the cells **40** are all aligned or focused along the wall. The cells **40** then continue downstream to the separation region **60** where, like the prior embodiment of FIG. **6**, the cells **40** represented by the solid circles are preferentially moved (in this case attracted) toward the DEP electrodes **26**. This causes the solid cells **40** to enter the upper branch channel **18***a* while the cells **40** represented by the open circles enter the lower branch channel **18***b*.

As an alternative to the use of MHD electrodes **52** to align or focus cells **40** or particles **44**, laminar flow may be used to set the cells **40** or particles **44** in a uniform "start" condition. For example, laminar flow may be used to provide a line of cells **40** or particles **44** along either wall of the microchannel **14**. Alternatively, sheath type flow may be used to confine the cells **40** or particles **44** within a centrally located region of the microchannel **14**.

FIG. **8** illustrates yet another embodiment of a device **10** that uses vertically oriented electrodes **26** to separate a heterogeneous population of cells **40** or particles **44**. The device in FIG. **8** operates in a "free flow" mode. In this regard, unlike the embodiments of FIGS. **6** and **7**, the population of cells **40** or particles **44** is not focused or aligned prior to separation. Instead the electrodes **26**, which may be DEP electrodes, are driven at different states to spatially separate the cells **40** or particles **44** that flow through the region of the microchannel **14** that contains the opposing set of electrodes **26**. For example, the DEP electrodes **26** on one side of the microchannel **14** may be driven at a first frequency while the DEP electrodes **26** on the opposite side of the microchannel **14** are driven at a second, different frequency. By driving the DEP electrodes **26** at different frequencies, the different cells **40** or

particles **44** can be spatially separated within the microchannel **40**. The voltage amplitudes may also be altered to effectuate spatial separation. In effect, each particular cell type or particle type has a unique "DEP spectra" which can be used to separate a heterogeneous population.

In the device **10** of FIG. **8**, the cells of a first type **40***a* preferentially move toward one side of the microchannel **14** while cells of another type **40***b* preferentially move toward the other side of the microchannel **14**. As the cells move between the electrodes **26** they begin to stratify along the width of the microchannel **14**. This spatial separation then causes the two cell types **40***a*, **40***b* to flow into different branch channels **18***a*, **18***b*. While the embodiment shown in FIG. **8** uses two cell types with two downstream channels, it should be understood that the "free flow" embodiment may be used within populations of cells **40** or particles **44** with more than two types. In this embodiment, the degree of separation is a function of the fluid flow rate as well as the length of the section containing the opposing DEP electrodes **26**. A slower flow rate will generally produce greater separation. In addition, more separation may be obtained by increasing the number of electrodes **26**.

FIG. **9** graphically illustrates how the device of FIG. **8** operates through the application of different frequencies/amplitudes to the DEP electrodes **26**. As seen in FIG. **9**, the vertically oriented DEP electrodes **26***a* on one of the side walls is driven at a frequency of 10 kHz while the opposing vertically oriented DEP electrodes **26***b* located on the other, opposing side wall is driven at 100 kHz. The voltage (AC) profiles of both sets of electrodes **26***a*, **26***b* are also illustrated. As seen in FIG. **9**, by driving the DEP electrodes **26***a*, **26***b* differently, cells **40***a* of a first type (e.g., cancer cells) are separated and later sorted into branch channel **18***a* while cells **40***b* of a second type (e.g., non cancerous cells) are separated and later sorted into the branch channel **18***b*.

A DEP-DEP "free flow" device like that shown in FIGS. **8** and **9** was tested for its ability to separate and sort cells **40** from microbeads **44**. A device **10** having opposing DEP electrodes **26** was used to separate polystyrene microbeads **44** and HEK **293** cells **40**. Separation was accomplished by using different driving frequencies for the opposing arrays of DEP electrodes **26**. Both cells **40** and microbeads **44** were introduced into the microchannel **14** at the same time. The cells **40** as shown in FIGS. **10**A and **10**B (shown circled by white colored dots) preferentially moved toward the upper DEP electrodes **26** while the microbeads **44** (shown circled by darkened dots) experienced attraction toward the lower set of DEP electrodes **26**. The cells **40** were then sorted into the upper branch channel **18***a* while the particles **44** were then sorted into the lower branch channel **18***b*.

FIG. **11**A illustrates the sorting percentage of the device **10** without the DEP electrodes **26** being powered. As seen in FIG. **11**A, the cells **40** and microbeads **44** were nearly equally distributed in the top and bottom branch channels **18***a*, **18***b*. In contrast, as seen in **11**B, when the DEP electrodes **26** were activated, the cells **40** were separated and sorted into the top branch channel **18***a* while the microbeads **44** were separated and sorted into the bottom branch channel **18***b*. This sorting device **10** and method is thus able to simultaneously discriminate and sort a heterogeneous population of cells **40** and particles **44**. This is important because the device **10** may have relatively high throughput levels.

FIGS. **12**A and **12**B illustrate an embodiment of a device **10** that uses DEP electrodes **26** to effectuate separation and sorting of cells **40** or particles **44** by trapping. In this device **10**, DEP electrodes **26** are positioned on one side of the microchannel **14**. The DEP electrodes **26** are driven such that cells **40** or particles **44** are temporarily trapped along the side wall of the microchannel **14**. For example, as shown in FIG. **12**A, cells **40***a* are trapped when the DEP electrodes **26** are energized while the cells **40***b* are not trapped and flow downstream in the fluid flow. The device **10** may include multiple branch channels **18***a*, **18***b* with each associated with a MHD valve **70**. The MHD valves **70** can be turned on or off to shunt fluid flow to a particular branch channel **18**. For example, in FIG. **12**A, the upper branch channel **18***a* is closed to permit the cells **40***b* to sort into the lower branch channel **18***b*. Once the cells **40***b* have entered the branch channel **18***b*, the MHD valve **70** associated with branch channel **18***b* is closed and the MHD valve for branch channel **18***a* is opened (as shown in FIG. **12**B). The trapped cells **40***a* can then be released by de-energizing the DEP electrodes **26**. The cells **40***a* are then free to flow into the other branch channel **18***a*.

The trapping aspect may also be integrated into "free flow" embodiments described herein. FIGS. **13**A and **13**B illustrate a device **10** and method that traps cells **40** or particles **44** in a free flow setting. In this embodiment, DEP electrodes **26** are positioned along one side wall of the microchannel **14**. The DEP electrodes **26** are capable of being driven so as to trap certain cells **40** or particles **44** passing through the microchannel **14**. Mixed populations of cells **40** (or particles **44**) are pumped into the microchannel **14**. When the DEP electrodes **26** are turned on as shown in FIG. **13**A, cells types **40***a* are trapped to the DEP electrodes **26**. Meanwhile the other type of cells **40***b* are repelled from the electrodes **26** and flow out the branch channel **18***b*. The trapped cells **40***a* are then released by turning off the DEP electrodes **26**. The cells **40***a* then naturally flow into the branch channel **18***a*. In this embodiment, there is no need for downstream valves or the like to aid in sorting the trapped cells **40**.

In addition, in another embodiment, the portion of the microchannel **14** that is downstream of the trapping DEP electrodes **26** may include a plurality of electrodes **26** that are used to selectively switch cells **40** or particles **44** into one or more selected branch channels **18**. In this regard, downstream electrodes **26** may be used to actively separate cells **40** or particles **44** into the appropriate branch channel **18**. For example, in this embodiment a cluster or group of cells **40** of a given type may be trapped while a cluster or group of cells **40** of another type are eluted passed the trapping DEP electrodes **26**. The eluted cells **40** may be actively moved into a particular branch channel **18** via a plurality of downstream-located electrodes **26**. The "trapped" cells **40** of the first type may then be released into the fluid flow. These formerly trapped cells **40** can then be actively guided via charged electrodes **26** into another branch channel **18** via the downstream-located electrodes **26**.

FIGS. **14**A and **14**B illustrate photographic images of a trapping device **10** like that illustrated in FIGS. **13**A and **13**B being used to trap and sort HEK293 cells **40** from N115 cells **40**. In FIG. **14**A, HEK293 cells are trapped on the DEP electrodes **26** (in FIG. **14**A the trapping is upstream and out of view of the image) while the N115 cells (circled by white colored dots) are repelled away from the DEP electrodes **26** and flow into the bottom branch channel **18***b*. FIG. **14**B illustrates the DEP electrodes **26** being turned off with the HEK293 cells (circled by black colored dots) were released from the DEP electrodes **26** and flow into the top branch channel **18***a*.

While embodiments of the present invention have been shown and described, various modifications may be made without departing from the scope of the present invention. The invention, therefore, should not be limited, except to the following claims, and their equivalents.

What is claimed is:

1. A microfluidic separation device comprising:

a first microchannel formed in a substrate, the first microchannel being defined by a bottom surface, a first wall, and second wall, the first microchannel including an upstream end and a downstream end;

a plurality of magneto-hydro dynamic electrodes disposed on the first wall configured to apply a substantially uniform force oriented generally perpendicular to a long axis of the first microchannel;

a plurality of dielectrophoretic electrodes disposed on the second wall opposite to the first wall configured to apply a substantially non-uniform force oriented generally perpendicular to a long axis of the first microchannel and opposite the force of the plurality of magneto-hydro dynamic electrodes;

a voltage source connected to the plurality of electrodes on the first wall and the second wall; and

a plurality of branch microchannels coupled to a downstream end of the first microchannel.

2. The device of claim **1**, wherein the first microchannel is configured to connect to a source of heterogeneous cells.

3. The device of claim **1**, wherein the first microchannel is configured to connect to a source of heterogeneous particles.

4. The device of claim **1**, further comprising a plurality of electrodes disposed on one of the first wall or the second wall at a position that is downstream of the opposing plurality of electrodes and upstream of the branch channels.

5. The device of claim **4**, wherein the plurality of electrodes disposed on one of the first wall or the second wall at a position that is downstream of the opposing plurality of electrodes and upstream of the branch channels is connected to a separate voltage source.

6. The device of claim **1**, further comprising a top surface covering the microchannel.

7. The device of claim **1**, wherein the plurality of branch channels include two or more branch channels.

8. A microfluidic separation device comprising:

a first microchannel formed in a substrate, the first microchannel being defined by a bottom surface, a first wall, and second wall, the first microchannel including an upstream end and a downstream end;

a focusing region disposed in the first microchannel comprising a plurality of electrodes disposed on the first wall and another plurality of electrodes disposed on the second wall opposite the plurality of electrodes disposed on the first wall;

a voltage source connected to the plurality of electrodes on the first wall and the second wall;

a separation region disposed in the first microchannel at a location downstream of the focusing region, the separation region comprising a plurality of magneto-hydro dynamic electrodes disposed on a first wall of the first microchannel and configured to apply a substantially uniform force oriented generally perpendicular to a long axis of the first microchannel and a plurality of dielectrophoretic electrodes disposed on a second wall of the first microchannel and configured to apply a substantially non-uniform force oriented generally perpendicular to a long axis of the first microchannel and opposite the force of the plurality of magneto-hydro dynamic electrodes of the first microchannel; and

a plurality of branch microchannels coupled to a downstream end of the first microchannel.

9. The device of claim **8**, wherein the first microchannel is configured to connect to a source of heterogeneous cells.

10. The device of claim **8**, wherein the first microchannel is configured to connect to a source of heterogeneous particles.

11. The device of claim **8**, wherein the plurality of electrodes disposed in the separation region are connected to a separate voltage source.

12. The device of claim **8**, further comprising a top surface covering the microchannel.

13. The device of claim **8**, wherein the plurality of branch channels include two or more branch channels.

14. A method of sorting particles or cells using a microfluidic device comprising:

providing a microfluidic channel having a bottom surface and two opposing side walls, the microfluidic channel having a focusing region comprised of a plurality of electrodes disposed on opposing side walls of the microchannel, the microfluidic channel further including a separation region located downstream of the focusing region and comprising a plurality of magneto-hydro dynamic electrodes disposed on one side of the side wall and a plurality of dielectrophoretic electrodes disposed on an opposing side of the side wall, the microchannel further including a plurality of branch channels located downstream of the separation region;

flowing a heterogeneous population of particles or cells within the microfluidic channel;

driving the plurality of electrodes in the focusing region to align the heterogeneous population of particles or cells within the microchannel;

driving the magneto-hydro dynamic electrodes to urge the heterogenous population of particles or cells toward the opposing dielectrophoretic electrodes;

driving the plurality of dielectrophoretic electrodes in the separation region so as to spatially separate the heterogeneous population of particles or cells; and

flowing the spatially separate heterogeneous population of particles or cells into the plurality of branch channels based at least in part on the spatial separation.

15. A method of sorting particles or cells using a microfluidic device comprising:

providing a microfluidic channel having a bottom surface and two opposing side walls, the microfluidic channel having a plurality of magneto-hydro dynamic electrodes disposed on one side of the microfluidic channel and a plurality of dielectrophoretic electrodes disposed on an opposing side of the microfluidic channel, the microfluidic channel further including a plurality of branch channels located downstream of the plurality of electrodes;

flowing a heterogeneous population of particles or cells within the microfluidic channel;

driving the magneto-hydro dynamic electrodes to urge the heterogenous population of particles or cells toward the opposing dielectrophoretic electrodes;

driving the plurality of dielectrophoretic electrodes so as to spatially separate the heterogeneous population of particles or cells; and

flowing the spatially separate heterogeneous population of particles or cells into the plurality of branch channels based at least in part on the spatial separation.

* * * * *